



The relationship between thermal physiology and lampricide sensitivity in larval sea lamprey (*Petromyzon marinus*)[☆]

Benjamin L. Hlina, Oana Birceanu¹, Christopher S. Robinson, Hadi Dhiyebi, Michael P. Wilkie^{*}

Department of Biology and Laurier Institute for Water Sciences, Wilfrid Laurier University, 75 University Avenue West, Waterloo, Ontario N2L 3C5 Canada

ARTICLE INFO

Article history:

Received 13 August 2020

Accepted 27 September 2021

Available online 27 October 2021

Communicated by Jessica Barber

Keywords:

3-Trifluoromethyl-4-nitrophenol

Invasive species

Fish

Biotransformation

Metabolism

Thermal optima

ABSTRACT

The sensitivity of larval sea lamprey (*Petromyzon marinus*) to the lampricide 3-trifluoromethyl-4-nitrophenol (TFM) varies with season, with highest sensitivity in spring and tolerance increasing by 2- to 3-fold in the mid-late summer. Until recently, the physiological basis for these differences was unresolved. Using previously published and unpublished findings, we illustrate how the acute toxicity of TFM (12-h LC₅₀, 12-h LC_{99.9}) changes with season in two populations of larval sea lamprey collected through the spring, summer and fall from Deer Creek and the Au Sable River, Michigan, U.S.A. Our findings reveal that the greater TFM tolerance of larval sea lamprey in the summer is most closely related to increases in water temperature. Although the energy reserves (glycogen, lipid) and body condition of larval sea lamprey may be lower in the spring after overwintering, these physiological indices have little impact on TFM sensitivity. We therefore conclude that water temperature, rather than energy stores or body condition, explains the greater tolerance of sea lamprey to TFM in the summer. We propose that as water temperature increases through the spring and summer, and approaches the thermal optima of larval sea lamprey, their metabolic rate and capacity to detoxify TFM increases, which slows the rate at which TFM accumulates in the body, despite concurrent increases in TFM uptake rate. We therefore recommend that water temperature be considered when planning and executing lampricide applications to mitigate temperature-induced increases in sea lamprey tolerance to TFM that could undermine sea lamprey control efforts in the Great Lakes.

© 2021 The Authors. Published by Elsevier B.V. on behalf of International Association for Great Lakes Research. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Introduction

The lampricide, 3-trifluoromethyl-4-nitrophenol (TFM) is a selective piscicide that has been used for over 60 years to control invasive sea lamprey (*Petromyzon marinus*) populations in the Great Lakes (Siefkes, 2017; Wilkie et al., 2019). Thought to have been originally restricted to the North Atlantic Ocean, anadromous sea lamprey invaded the Great Lakes in the early 1900s following the construction of shipping canals linking the basin to the eastern

seaboard of North America (see Lawrie, 1970; Eshenroder, 2009, 2014 for reviews). Sea lamprey spend most of their life, typically 3–7 years, burrowed in the substrate of freshwater streams as filter-feeding larvae, before undergoing a complex metamorphosis into parasitic/predatory juveniles which feed on the blood of fishes (Youson, 2003; Manzon et al., 2015). The effects of lamprey parasitism, combined with overharvest, eventually resulted in massive declines of culturally and economically important fish populations in the Great Lakes by the mid-20th century (Smith and Tibbles, 1980; Siefkes, 2017; Wilkie et al., 2021).

Sea lamprey populations were eventually brought under control after the implementation of a binational sea lamprey control program managed by the Great Lakes Fishery Commission (GLFC; GLFC, 2011) with TFM as the backbone of the control program. TFM selectively targets larval sea lamprey in infested tributaries due to their limited capacity to detoxify TFM compared to most non-target fishes (Lech and Statham 1975; Kane et al., 1994). Infested rivers and streams are usually treated with TFM on a 2–4 year cycle, with mortality typically exceeding 90 % (Barber and Steeves, 2021). The amount of TFM applied to streams is based

[☆] Given their role as Guest Editor, Michael Wilkie had no involvement in the peerreview of this article and had no access to information regarding its peer-review. Full responsibility for the editorial process for this article was delegated to Jessica Barber.

This article is published as part of a supplement sponsored by the Great Lakes Fishery Commission.

^{*} Corresponding author.

E-mail address: mwilkie@wlu.ca (M.P. Wilkie).

¹ Current Address: Department of Physiology and Pharmacology, Schulich School of Medicine and Dentistry, The University of Western Ontario, 1151 Richmond St., London, Ontario, N6A 3K7

on the minimum lethal concentration (MLC), the concentration of TFM needed to eradicate 99.9% of the larval sea lamprey over 9–12 h, at a given water pH and alkalinity (Bills et al., 2003; Barber and Steeves, 2021).

Exposure to TFM results in the uncoupling of mitochondrial oxidative phosphorylation (Niblett and Ballantyne, 1976; Birceanu et al., 2011; Huerta et al., 2020), which creates a mismatch between ATP supply and demand (Birceanu et al., 2009; Clifford et al., 2012; Ionescu et al., 2021). In sea lamprey and non-target fishes such as the rainbow trout (*Oncorhynchus mykiss*) and lake sturgeon (*Acipenser fulvescens*), this forces the animals to rely on anaerobic energy reserves, such as glycogen and phosphocreatine, to make-up for the shortfalls in aerobic ATP production. The anaerobic energy stores are finite, however, and death ensues when ATP demands can no longer be met (Wilkie et al., 2007; Birceanu et al., 2009; Clifford et al., 2012; Ionescu et al., 2021). Although TFM treatments result in high mortality in larval sea lamprey, “residual sea lamprey” that survive treatment remain an ongoing challenge for sea lamprey control, and are a major source of parasitic juvenile sea lamprey if left untreated (Barber and Steeves, 2021). It is therefore important to identify factors that explain variation in the sensitivity of larval sea lamprey to TFM.

Recent work has shown that differences in the sea lamprey's responses to TFM are related to biotic factors, such as life stage and body size (Henry et al., 2015; Tessier et al., 2018). However, environmental variables such as water pH and alkalinity also alter TFM speciation and rates of TFM accumulation (Hunn and Allen, 1974; McDonald and Kolar, 2007; Hlina et al., 2017; reviewed by Wilkie et al., 2019). TFM is a weak acid, with an ionizable hydroxyl group (–OH), having a pKa between 6.07 and 6.38 (Hubert, 2003; McConville et al., 2016). As pH increases, the proportion of TFM-OH decreases and less lipid soluble TFM-O[−] increases, leading to slower overall rates of TFM uptake at a given concentration of TFM. For this reason, the amounts of TFM needed to eradicate larval sea lamprey (e.g., 12-h LC₅₀) increase as water pH goes up (Bills et al., 2003; Wilkie et al., 2021).

Similar to pH, TFM requirements increase with alkalinity (Bills et al., 2003), likely because the water buffering capacity is higher, which limits how much the water pH at the gill surface (gill microenvironment) is lowered (acidified) due to the excretion of CO₂ and metabolically derived acid across the gills (Playle and Wood, 1989; Erickson et al., 2006). As a result, less TFM is in its more bioavailable TFM-OH form at the gill surface, the putative site of TFM uptake, necessitating the addition of higher amounts of TFM in higher alkalinity waters (reviewed by Wilkie et al., 2021).

One environmental variable that has received little attention is water temperature. A few studies have indicated that the tolerance of sea lamprey to TFM varies with season, peaking in the mid-late summer, before declining in the fall (Fig. 1; Scholefield et al., 2008; Muhametsafina et al., 2019). Sea lamprey control agents typically compensate for seasonal variations in TFM effectiveness, by applying TFM at less than MLC in the spring and greater than MLC in the summer (B. Morrison, Sea Lamprey Control Centre, Fisheries and Oceans Canada, Sault. Ste. Marie, ON, pers. communication). However, an understanding of the underlying physiological causes of seasonal variation in TFM sensitivity had been lacking.

Recently, we explored how seasonal variation in the TFM sensitivity of larval sea lamprey were related to changes in their body condition and internal energy stores, and with water temperature through the spring, summer and fall (Muhametsafina et al., 2019). Notably, we found that maximal TFM tolerance coincided with peak water temperatures, but not condition factor or internal energy stores (Muhametsafina et al., 2019). The goals of the present paper are to: (1) build on this earlier work, along with new data, to demonstrate that seasonal variation in TFM sensitivity is mainly a function of water temperature, and not body condition

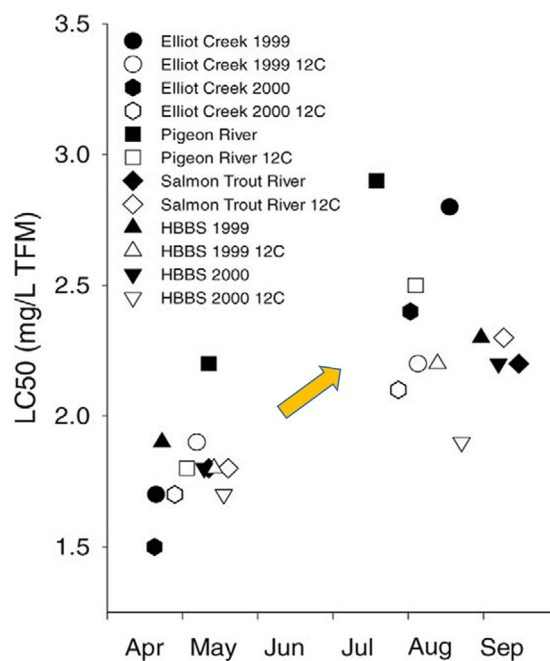


Fig. 1. Changes in TFM sensitivity with season in sea lamprey. Differences in the 9-h LC₅₀ of TFM to larval sea lamprey collected from different streams draining into Lake Huron or Lake Michigan in April and May, and then again from July to August. Toxicity was assessed using flow through acute toxicity tests at either 12 °C or at seasonal temperatures, at the Hammond Bay Biological Station, Millersburg, MI. Note the increase in the 9-h LC₅₀ observed between the spring and summer for each data set. From Scholefield et al. (2008) with permission.

or internal energy stores; (2) use our knowledge of the thermal physiology of larval sea lamprey to explain why larval sea lamprey TFM tolerance increases in warmer waters; (3) describe how a better knowledge of the thermal physiology and TFM detoxification capacity of sea lamprey can be used to more accurately predict how the toxicity of TFM to sea lamprey changes at different water temperatures; (4) propose how TFM application procedures can be modified to account for changes in the TFM sensitivity of sea lamprey with temperature; and (5) suggest how water temperature might be incorporated into predictive models, similar to the pH-alkalinity model, to more accurately predict how TFM application rates and overall consumption change seasonally.

Method and materials

Experimental animals and holding

All original data presented in this paper were generated using larval sea lamprey (N ~ 1,000) captured by pulsed-DC electrofishing (ABP-2 Electrofisher, Electrofishing Systems, LLC, Madison, WI, USA) from Deer Creek, Michigan in late April-early May, June, August and September 2011 by United States Fish and Wildlife Service personnel (Ludington, MI USA) and transported in well oxygenated water in coolers to the Hammond Bay Biological Station (HBBS; United States Geological Survey, Millersburg, MI, USA). In all cases, animals were held in aquaria continuously receiving aerated Lake Huron water (HBBS), maintained at the same temperature (±1°C) as Deer Creek. Each tank was filled with sand to a depth of approximately 5 cm to provide burrowing substrate for the larva, and the animals were not fed as experiments were conducted within 5–10 d of capture. All experiments were approved by the Wilfrid Laurier University Animal Care Committee (Animal Use Protocol Number: R10004) and followed Canadian Council of Animal Care guidelines.

Experimental protocols

Effects of season on TFM toxicity

To ascertain how season (spring, early and late summer, fall) affected the TFM sensitivity of larval sea lamprey, we conducted acute toxicity tests to determine the 12-h LC_{50} and 12-h $LC_{99.9}$ at each time of the year (e.g., Muhametsafina et al., 2019). Each acute toxicity test was preceded by a smaller scale, range-finder test to estimate the TFM concentration range that caused mortality. This information was then used to select the concentrations used to determine the actual 12-h LC_{50} and 12-h $LC_{99.9}$ in the larger scale acute toxicity tests that followed. In each range-finder test, groups of lamprey ($N = 10$ per concentration) were exposed to nominal TFM concentrations of 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0 $mg\ L^{-1}$ in glass aquaria containing 16 L of Lake Huron water (pH 7.8), plus unexposed controls ($N = 10$). Based on the outcomes of these range-finder tests, six TFM concentrations were selected for the subsequent larger scale acute toxicity tests using a similar design, but larger volume of water (~ 30 L; Table 1). The animals were exposed to each concentration of TFM in duplicate or triplicate ($N = 10$ –15 per aquarium), plus one tank containing unexposed controls. The tests followed American Standard and Testing Methods guidelines (ASTM, 2007) using field grade TFM (35 % active ingredient dissolved in isopropanol; Clariant SFC GMBH WERK, Griesheim, Germany) provided courtesy of the HBBS.

Approximately 12 h before each range finder or acute toxicity test, the appropriate amounts of TFM were added to glass aquaria filled with continuously aerated Lake Huron water of the appropriate temperature. After 10 min of mixing, and again the next morning before addition of test animals to the aquaria, 10 mL water samples were collected for measurement of TFM concentration (see Analytical techniques below). Temperature was maintained by immersion of the aquaria in a temperature-controlled water bath. Mortalities were monitored hourly for the first 12 h, and again at 24 h, but only 12-h LC_{50} s and 12-h $LC_{99.9}$ s are reported. If mortality was suspected, based on lack of movement or ventilation, the tail was lightly pinched with tweezers, followed by the immediate removal of unresponsive (dead) animals. Those that were still alive at 24 h were euthanized using tricaine methanesulfonate (1.5 $g\ L^{-1}$ MS-222 buffered with 3.0 $g\ L^{-1}$ $NaHCO_3$; Syndel

Labs, Nanaimo, BC, Canada). The 12-h and 24-h LC_{50} and $LC_{99.9}$ were calculated using Probit Analysis (see Statistical analysis below). Immediately after removal from their respective tanks, each dead or surviving animal was blotted dry with a paper towel, followed by measurements of the body length and mass.

Effects of season on body energy stores

Sub-sets of animals ($N = 48$) collected from Deer Creek (2011) in May, June, August and September, were transferred to 1 L darkened plastic containers ($n = 12$ containers; $n = 4$ larvae per container in triplicate) receiving Lake Huron water at a rate of $\sim 150\ mL\ min^{-1}$ at the appropriate temperature. Diffuse cotton (2 g per container) was added to each container to act as burrowing substrate to calm the larvae (Wilkie et al., 2001; Birceanu et al., 2009). After an overnight acclimation period, the water level in each container was reduced to 750 mL, followed by the addition of an anesthetic dose of MS-222 (0.5 $g\ L^{-1}$; buffered with 1.0 $g\ L^{-1}$ of $NaHCO_3$), and then a lethal dose of MS-222 (1.5 $g\ L^{-1}$; buffered with 3.0 $g\ L^{-1}$ of $NaHCO_3$). Tissues (brain, liver and carcass) were immediately collected, freeze-clamped using liquid nitrogen cooled aluminum tongs, and stored at $-80\ ^\circ C$ until analyzed for glycogen, protein, lipid, ATP, phosphocreatine, lactate, dry ash, and water content.

Analytical techniques

Water TFM analysis

The TFM concentration of water samples used in toxicity tests was determined spectrophotometrically using a Genesys 6 spectrophotometer (at HBBS; Thermo Electron Corporation, MA USA) and precision standards at a wavelength of 395 nm according to standard operating procedures (e.g. IOP 012.4; Sea Lamprey Control Centre, Fisheries and Oceans Canada, Sault Ste. Marie, ON, Canada).

Tissue processing for energy stores

All enzymes and reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA), unless otherwise stated. Tissue (muscle and brain) processing for glycogen, glucose, ATP and phosphocreatine is outlined in Birceanu et al. (2009, 2014). Briefly, frozen muscle pieces were pulverized under liquid nitrogen into a fine powder

Table 1

Water quality and TFM concentrations to which larval sea lamprey collected from Deer Creek, Michigan were exposed during measurements of acute TFM toxicity in May, June, August and September 2011.

Month	Temp ($^\circ C$)	pH	Nominal [TFM] ($mg\ L^{-1}$)	Measured [TFM] ($mg\ L^{-1}$)
May	11.6 ± 0.02^a	7.74 ± 0.01^a	0.7	0.78 ± 0.01
			1.1	1.25 ± 0.05
			1.3	1.45 ± 0.05
			1.5	1.55 ± 0.03
			1.7	1.65 ± 0.03
			2.0	1.86 ± 0.03
			1.7	1.68 ± 0.01
June	18.0 ± 0.03^b	7.85 ± 0.02^b	2.0	2.09 ± 0.0
			2.3	2.38 ± 0.01
			2.5	2.58 ± 0.01
			2.7	2.75 ± 0.04
			3.0	3.09 ± 0.01
			2.7	2.80 ± 0.02
			3.3	3.42 ± 0.0
August	22.2 ± 0.02^c	8.04 ± 0.02^c	3.7	3.86 ± 0.02
			4.1	4.26 ± 0.0
			4.5	4.69 ± 0.01
			5.0	5.21 ± 0.01
			1.5	1.47 ± 0.02
			2.0	2.00 ± 0.01
			2.3	2.31 ± 0.01
September	11.8 ± 0.03^a	8.15 ± 0.02^d	2.6	2.64 ± 0.01
			3.0	3.08 ± 0.01
			3.5	3.60 ± 0.01

using a mortar and pestle. Next, four parts of homogenization buffer (8% perchloric acid, 1 mmol L⁻¹ EDTA) were added to the homogenate, which was placed on ice for 10 min, with mixing every 2–3 min. Then, the sample was split into two aliquots: one was designated for tissue glycogen measurements and was neutralized with 3 mol L⁻¹ K₂CO₃, snap frozen in liquid nitrogen, and kept at -80 °C for later analysis; the second aliquot, designated for ATP and phosphocreatine, was centrifuged at 10,000g for 2 min at 4 °C, the supernatant drawn off, weighed and neutralized with 2 mol L⁻¹ KOH cocktail (0.4 mol L⁻¹ each of imidazole and KCl). This solution was mixed again using a vortex mixer, centrifuged at 10,000g for 2 min at 4 °C, and the supernatant flash frozen in liquid nitrogen and stored at -80 °C for later analysis. Brain samples were processed identically, except that after the tissue was weighed, the homogenization buffer was added straight to the microcentrifuge tube and the sample was homogenized using a handheld motorized pestle (Gerresheimer Kimble Kontes LLC, Dusseldorf, Germany).

Measurements of tissue glycogen, ATP and phosphocreatine were made as described in [Muhametsafina et al. \(2019\)](#). For glycogen analysis, the first aliquot of the homogenate containing tissue glycogen was broken down into glucose using amyloglucosidase (40U per sample) in acetate buffer (2 mmol L⁻¹, pH 4.5) at 37 °C for 2 h and the reaction terminated by the addition of 70% perchloric acid, followed by neutralization with 3 mol L⁻¹ K₂CO₃. Samples were stored at -80 °C for later enzymatic determination of glucose concentration using hexokinase. Levels of ATP and phosphocreatine were measured enzymatically in the second aliquot as described in [Birceanu et al. \(2014\)](#) and [Muhametsafina et al. \(2019\)](#). Glucose, glycogen, ATP and phosphocreatine concentrations were expressed as μmol g⁻¹ wet tissue mass.

Tissue processing for lipid, protein, water and dry ash measurements

Carcasses (body without the brain and the liver) were ground to a fine powder under liquid nitrogen using a mortar and pestle, and separate aliquots set-aside for lipid, protein, water and dry ash determination. Carcass lipid content was determined gravimetrically using the chloroform: methanol extraction method ([Lauff and Wood, 1996](#)). Approximately 100 mg ground tissue was added to 10 mL of chloroform: methanol (2:1) solution in a 20 mL glass scintillation vial, mixed and incubated at 4 °C for 12 h. After incubation, 2.6 mL of 0.9% NaCl solution was added to each vial; the samples were mixed and incubated again for 12 h at 4 °C. Next, a 5-mL syringe fitted with a 25-gauge needle was used to collect and transfer the 4-mL chloroform phase (containing the lipids) into a pre-weighed glass culture tube. The chloroform was then evaporated to dryness under nitrogen gas. The weight of the culture tubes plus the remaining residue was then recorded. Lipid was calculated as the difference between the weight of the tube plus residue and the weight of the same, clean tube.

Protein concentrations were determined using the Bradford assay ([Bradford, 1976](#)), with bovine serum albumin as the standards. Ground tissue was weighed and 4 parts Tris-HCl buffer (pH 7.4) was added. A hand-held homogenizer was used to further breakdown the tissue and the homogenate was diluted 50 times before protein analysis. Protein levels were determined in a 96-well plate using a plate spectrophotometer (Epoch 2; BioTek, Winooski, VT, USA).

Water content was determined gravimetrically by transferring approximately 50 mg aliquots of the ground tissue powder to a pre-weighed microcentrifuge tube, measuring the wet tissue mass, and then drying the samples to constant mass at 60 °C for 48 h. Whole body water content was based on the percent difference between the wet and dry mass of the powder and expressed as % tissue water. The dried tissue was then transferred to tared

crucibles and combusted (ashed) at 750 °C for 4 h, with the amount of ash expressed per unit wet mass (g wet mass⁻¹).

Calculations and statistics

Condition factor (CF) for each larval sea lamprey was calculated according to [Holmes and Youson \(1994\)](#) using the following equation:

$$CF = \left[(M) / (L^3) \right] \times 10^6 \quad (1)$$

where *M* denotes the wet mass of the lamprey (g) and *L* is the total length measured from the tip of the snout to the terminal ending of the caudal fin (mm).

Quantification of TFM toxicity was based on calculations of the 12-h median lethal concentration of TFM needed to cause death in 50 % of the test animals (12-h LC₅₀), the minimum lethal concentration required to kill 99.9 % of the animals (MLC), and median lethal time to lethality for 50 % of sea lamprey when exposed to a given concentration of TFM (LT₅₀). Briefly, the TFM exposure concentration (mg L⁻¹) was log₁₀ transformed and the proportion of animals that experienced mortality during the 12 h exposures time were calculated. Once calculated, both variables were used in functions that run binomial Generalized Linear Models with a probit link transformation which are found in the R package 'ecotox', v1.4.2, available on CRAN.

All physiological data are presented as the mean ± 1 standard error of the mean (SEM). Normality of the data was first assessed using Shapiro-Wilk test, kurtosis and skewness ('moments' v0.14) and homoscedasticity was assessed using a Levene's test ('car' v3.0-3). If the data met assumptions of a parametric test, a parametric test was used. When data did not meet the assumptions of the chosen parametric test it was either log₁₀ transformed or power transformed and reassessed for normality and homoscedasticity. If the transformations of the data did not correct for normality and homoscedasticity that data was analyzed using a non-parametric test (e.g., Kruskal-Wallis rank sum test). Carcass glycogen and whole body protein data versus season were analyzed using a one way ANOVA followed by a Tukey's honestly significant difference (HSD) test while brain glycogen data versus season were analyzed using a Welch's one-way analysis of means (not assuming equal variances) followed by a Games-Howell post hoc test ('userfriendlyscience' v0.7.2). Brain and carcass ATP, PCr data and whole body lipid, dry ash, and water content data versus season were analyzed using a Kruskal-Wallis rank sum test, followed by Dunn's post-hoc test. For all statistical tests, the level of significance was set at a *P* ≤ 0.05 and only non-transformed data are shown in figures. Statistical analysis were completed using R version 3.5.3, RStudio version 1.1.456 ([RStudio, 2018](#)), and 'ggplot2' ([Wickham, 2016](#)), ISBN: 978-3-319-24277-4.

Results and discussion

Effectors of TFM sensitivity in larval sea lamprey

The TFM sensitivity of sea lamprey changes with season

[Applegate et al. \(1961\)](#) first reported that larval sea lamprey tolerance to TFM varies seasonally, with greatest toxicity in late fall, winter and early spring, and lowest toxicity in mid- and late summer. They also noted that the amount of variation was dependent upon location within the Great Lakes, with seasonal differences being negligible in some waters and very pronounced in others suggesting that a range of potential abiotic and biotic factors interacted to influence toxicity. [Scholefield et al. \(2008\)](#) later demonstrated that time of year plays a critical role in how the sea lamprey respond to TFM exposure, reporting that the 9-h LC₅₀

and LC_{99.9} in the summer (July–August) were approximately 1.5-fold greater than in spring (May–June) in several Michigan streams (Fig. 1).

More recently, Muhametsafina et al. (2019) noted similar seasonal variation in TFM sensitivity in larval sea lamprey collected from the Au Sable River, Michigan, with greatest tolerance in the summer. Similar results were observed in Deer Creek, Michigan, which was also sampled throughout the year corroborating these observations (Fig. 2). Similar to the Au Sable River study (Muhametsafina et al., 2019), larval sea lamprey were collected from Deer Creek on four occasions (May, June, August, September), and TFM sensitivity was determined by performing acute toxicity tests within 7–10 days of capture, under near identical temperatures to those at the time of capture (Fig. 2A). As the corresponding concentration–response (dose–response) curves illustrate, sensitivity was greatest in the spring, when water temperatures were coolest, peaking in later summer (August) when water temperatures were near 25 °C, before declining in the fall when water temperatures were approaching spring-time values between 10 and 15 °C (Fig. 2A, B). When the data used to generate the toxicity curves were expressed as 12-h LC₅₀ or 12-h LC_{99.9} values using log probit analysis, it was apparent that the larval sea lamprey collected in summer were 2.5–3.0 fold more tolerant to TFM than the animals captured in the spring, and about 2-fold more tolerant than those captured in the fall (Fig. 2C).

Both the Deer Creek and Au Sable River (Muhametsafina et al., 2019) studies were limited to one season and one river, but we measured TFM toxicity on freshly caught sea lamprey (within 7–10 days) at multiple periods through the lampricide treatment season, which would minimize variation in TFM sensitivity due to thermal regime, food availability, and/or water quality. A limitation of this approach is that we cannot say with certainty that the trends would be the same from year to year or between different streams and rivers across the Great Lakes basin. It would therefore be informative to examine in more depth the seasonal and spatial variation in TFM sensitivity over multiple seasons, years and locations throughout the Great Lakes basin. Nevertheless, the weight of evidence from this work and the earlier studies clearly indicated that TFM tolerance varies seasonally in larval sea lamprey.

Differences in water pH can be ruled out as the primary cause of the variation in TFM toxicity observed with season in both the Au Sable River and Deer Creek studies. Water pH did vary in the Deer Creek study, increasing from a low of 7.74 in May to 8.15 in September (Table 1), but the changes in toxicity were much greater than predicted by pH alone. Assuming that the alkalinity of the water was 90 mg CaCO₃ L⁻¹ (K. Slaght, HBBS, unpublished data), the MLC measurements reported in the Deer Creek study were higher than the predicted MLC values by 1.1 mg L⁻¹ in May, 1.7 mg L⁻¹ in June, 4.4 mg L⁻¹ in August and 0.9 mg L⁻¹ in September, which were well in excess of the pH-dependent differences predicted using the tables published by Bills et al. (2003; Table 2). This was even true when the MLC chart values were multiplied by 1.4 times, which is a more realistic approximation of the concentrations of TFM that would be applied to a sea lamprey-infested stream. Similarly, Scholefield et al. (2008) reported that the MLC of TFM (9-h LC_{99.9}) in the summer were 1.3- to 1.7-fold higher than those predicted by the pH and alkalinity charts, with only spring MLC measurements similar to those provided by the charts.

The patterns of lethality in the sea lamprey collected from Deer Creek, as depicted by our measures of the median time to lethality (LT₅₀), were also informative because it allowed us to study the patterns of mortality and survival over 24 h, which encompasses the duration of a typical lampricide treatment (9–12 h). In many cases, split-probits, in which there are abrupt changes (inflections) in the slope of the probit mortality vs log time relationship, were

observed and these were more prevalent in the summer compared to the spring and fall, which were more linear (Fig. 3; Table 3). Split-probits typically indicate a change in the mode of toxicity, due to the accumulation of toxic metabolites in the animals (Sprague 1969; Newman and Unger 2003). On the other hand, if the slope of the probit mortality vs log time relationship flattens, which was particularly notable in August (Fig. 3C), it may be indicative of acclimation to the toxicant and/or development of resistance to the chemical, as is often the case with insects exposed to pesticides (Sprague 1969).

Energy reserves and condition have little influence on TFM sensitivity

The overwintering period for sea lamprey, like many other fishes, is characterized by cold water temperatures and a lack of food and corresponding reductions in energy reserves and body condition (O'Boyle and Beamish, 1977; Swink and Johnson, 2014). To determine if and how these variables influenced TFM sensitivity, we collected brain, liver and muscle from a subset of the larval sea lamprey collected from Deer Creek at each sample period, followed by analysis of tissue ATP, phosphocreatine, glycogen, lactate, lipid, protein, and water content.

Glycogen reserves in the liver of larval sea lamprey are low compared to other vertebrates (O'Boyle and Beamish, 1977). These low liver glycogen concentrations are offset, however, by the much higher glycogen concentrations in the meninges of the brain (Rovainen, 1970; Rovainen et al., 1971; Foster et al., 1993; Clifford et al., 2012; Henry et al., 2015), which provide it with the glucose needed to fuel central nervous system activity (see Polakof et al., 2012 for review). In rainbow trout (*Oncorhynchus mykiss*), brain glycogen reserves are reduced following fasting or hypoglycemia, underscoring glycogen's importance in providing glucose for sustaining brain function (Soengas et al., 1998; Polakof et al., 2012). We observed significantly lower brain glycogen concentration during the spring in Deer Creek sea lamprey (Fig. 4A), which is consistent with a period of prolonged food limitation during the winter months. This does not, however, explain the greater TFM sensitivity we observed in the spring, because TFM tolerance decreased again in the fall (Fig. 2C), when brain glycogen concentrations were much higher, and similar to the concentrations measured in June or August when TFM tolerance was greatest. Carcass glycogen was less than 4 μmol g⁻¹ wet mass in the larvae sampled in May, which we speculate was also due to prolonged food limitation during the overwintering period (Fig. 4A). These values were much lower than previous measurements made by O'Boyle and Beamish (1977), who noted that muscle glycogen peaked near 40 μmol g⁻¹ wet mass in spring and early summer. However, carcass glycogen concentrations were comparable in the two studies between June and August, suggesting that the Deer Creek lamprey had replenished their reserves by this time.

It seems unlikely that the much lower glycogen measured in the sea lamprey during the spring contributed to greater TFM sensitivity for two reasons. First, like many fishes, larval sea lamprey normally maintain higher glycogen stores in the muscle which is used as an energy resource that can be rapidly mobilized when ATP demands increase for burrowing or other forms of burst exercise (Wilkie et al. 2001). Second, the muscle has very low activities of the enzyme glucose-6-phosphatase, which is required to convert the glucose-6-phosphate generated from glycogen into glucose, and would therefore make little contribution to maintaining glucose homeostasis and central nervous system function (Panserat et al., 2000). It was also notable that over-wintering had no effect on lowering brain ATP or phosphocreatine (PCr), which buffers ATP supplies in the body through the creatine phosphokinase reaction (Fig. 5A, B). There was greater variability in carcass ATP, but the physiological relevance is unclear, as levels were not unusually low.

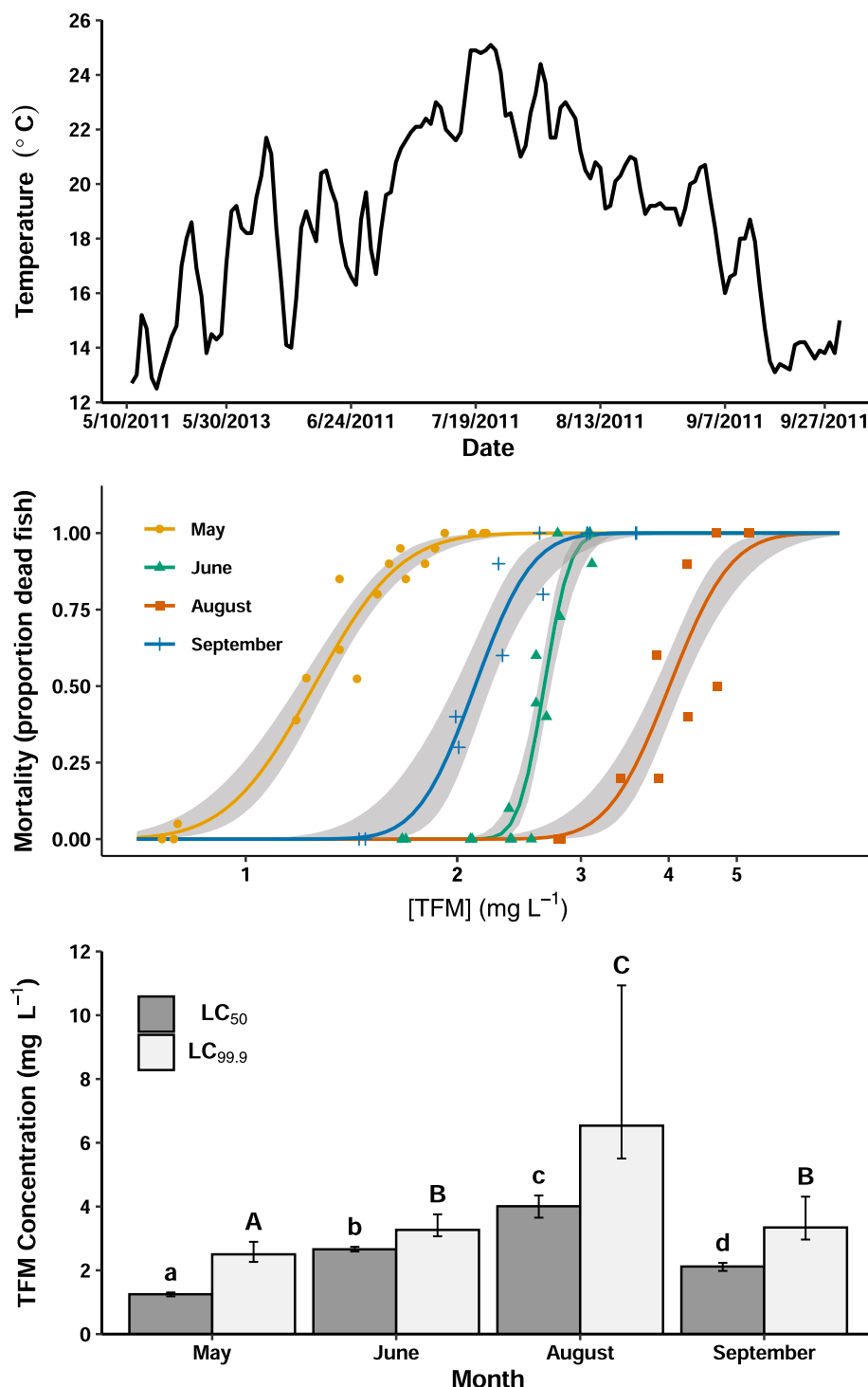


Fig. 2. Seasonal differences in the toxicity of 3-trifluoromethyl-4-nitrophenol (TFM) to larval sea lamprey. (A) Changes in the temperature of Deer Creek, Michigan from May–September 2011 recorded using an in-stream temperature logger (minilog-T, Vemco, Inc). (B) Dose-response curves $\pm 95\%$ confidence intervals (shaded regions) describing the changes in the TFM-induced lethality experienced by larval sea lamprey collected from Deer Creek Michigan in May, June, August and September 2011, and (C) the corresponding 12-h LC_{50} and minimum lethal concentration (R package ‘ecotox’ v1.4.2; 12-h $LC_{99.9}$; MLC) of TFM $\pm 95\%$ confidence interval in the same larvae. Statistical significance was determined by evaluating if confidence intervals overlapped.

The relatively low concentration of lipid and glycogen stores measured in larval sea lamprey carcasses in early May followed by a rapid accumulation of these reserves in June are also in agreement with previous studies addressing how energy stores and body composition (water content, dry ash) change through the year in non-metamorphosing and metamorphosing sea lamprey

(Fig. 4B, Table 4; Lowe et al., 1973; O’Boyle and Beamish, 1977). Notably, protein concentrations were similar across seasons (Fig. 4C). Protein would have been expected to be much lower if the animals were in an advanced state of starvation (Pottinger et al., 2003). The combination of low food availability and inefficient assimilation of nutrients (lipid, carbohydrate, protein), which

Table 2

Differences in the observed and predicted minimum lethal concentration (MLC) of TFM to larval sea lamprey collected from Deer Creek, MI in May, June, August, and September of 2011. Each seasonal test was conducted at ambient stream temperature and pH, in Lake Huron water. Different superscript letters for values of Observed MLC indicate significant statistical differences. Observed MLC data (plus upper and lower confidence intervals) also presented in Fig. 1. Predicted 12-h MLC values taken from charts depicting expected lethal concentrations of TFM producing 99.9% mortality in larval sea lamprey at selected pH and alkalinity values (Bills et al. 2003). Water pH data taken from Table 1 and alkalinity of Lake Huron water at Hammond Bay Biological Station assumed to average 90 mg CaCO₃ L⁻¹. The predicted MLC multiplied by 1.4 times (1.4 X), which is equivalent to the amounts often applied during field applications of TFM.

Month	pH	Observed MLC (12-h LC _{99.9})	Predicted MLC (12-h LC _{99.9})	1.4 X Predicted MLC
May	7.74	2.50 ^a (2.26–2.89)	1.4	1.96
June	7.85	3.27 ^b (3.07–3.76)	1.6	2.24
August	8.04	6.54 ^c (5.51–10.90)	2.1	2.94
September	8.15	3.34 ^b (2.97–4.31)	2.4	3.36

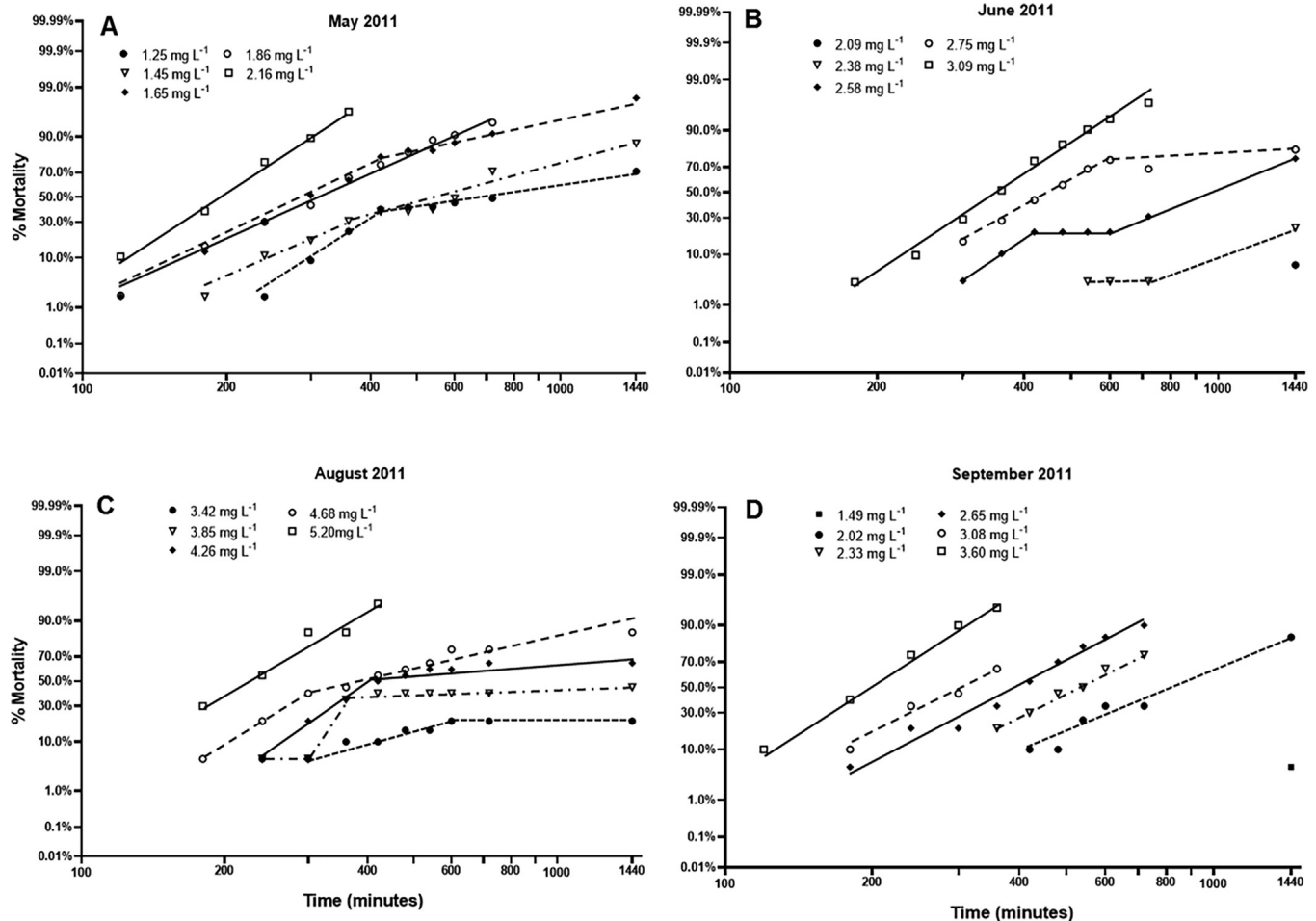


Fig. 3. Relationship between season and time to lethality during TFM exposure. Time versus lethality relationship in larval sea lamprey exposed to different concentrations of TFM captured from Deer Creek, MI, in (A) May, (B) June, (C) August and (D) September 2011. The exposure concentrations of TFM used for this analysis were those used to determine the 12-h LC₅₀ and LC_{99.9} in Fig. 2. Data depicted as % mortality, on a probit scale, plotted against time (24 h) on a logarithmic scale. Lines of best fit were determined by non-linear regression (Prism 8.03, Graphpad Software, La Jolla, CA), and used to determine the corresponding LT₅₀ values ($\pm 95\%$ CI) values (Table 2). N = 53–57 per test concentration in May, N = 28–31 in June, and N = 20 in August and September.

are much lower in cold (e.g., 5 °C) compared to warmer (e.g., 15 °C) waters, likely explained the depletion of lipid stores (Moore and Mallatt, 1980). These findings are consistent with greater reliance on lipid catabolism over the over-wintering period, when food resources were likely scarce in Deer Creek, followed by rapid lipid accretion when food availability increased in spring and early summer. While these observations might explain the significantly lower mass, length, and condition factor that were observed in spring compared to the summer months (Table 5), this too is unlikely to explain the greater TFM tolerance of larval sea lamprey in summer. Although the condition factor and energy reserves were

reduced in the spring, they do not appear to be a proximate effector of TFM sensitivity. However, it should be kept in mind that we only measured proximate body composition over one field season. Further studies over multiple years are needed to determine with greater certainty if changes in energy stores significantly affect the tolerance of larval sea lamprey to TFM.

Another possibility that can likely be discounted, is that larval sea lamprey with higher body masses were more tolerant to TFM due to lower mass specific rates of TFM uptake compared to smaller animals (Tessier et al., 2018; Wilkie et al., 2021). Due to the relatively small differences in larval sea lamprey body size detected in

Table 3

Relationship between time to lethality (LT_{50}), TFM concentration and season in larval sea lamprey. LT_{50} , the time to 50% mortality during exposure to a given concentration of TFM, was calculated for each dataset using non-linear regression to generate curves of best-fit. $N = 53$ –57 per test concentration in May, $N = 28$ –31 in June, and $N = 20$ in August and September.

May			June			August			September		
[TFM]	LT_{50}	95% CI	[TFM]	LT_{50}	95% CI	[TFM]	LT_{50}	95% CI	[TFM]	LT_{50}	95% CI
($mg\ L^{-1}$)	(min)		($mg\ L^{-1}$)	(min)		($mg\ L^{-1}$)	(min)		($mg\ L^{-1}$)	(min)	
0.78	None	–	1.67	None	–	2.80	None	–	1.49	1721.0	–
1.25	685.8	587.8–840.3	2.09	1685.0	–	3.42	4967.0	2680–19855	2.02	818.0	750.2–901.0
1.45	568.6	529.9–615.6	2.38	2167.0	2020.0–2344.0	3.85	1014.0	685.9–2393	2.33	525.4	506.0–546.4
1.65	301.6	290.4–312.9	2.58	939.5	860.4–1038.0	4.26	506.5	426.3–651.8	2.65	399.4	384.6–414.1
1.86	308	300.8–315.1	2.75	465.0	426.7–511.0	4.68	403.6	373.4–436.5	3.08	292.3	269.7–314.6
2.16	193.6	190.0–197.2	3.09	353.6	350.3–357.0	5.20	223.4	214.1–232.8	3.60	193.6	191.2–196.2

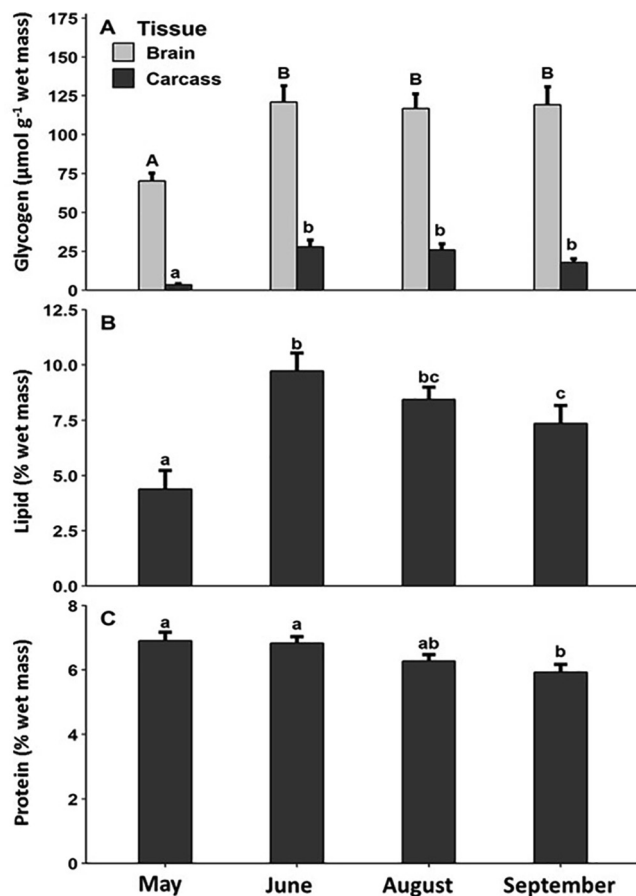


Fig. 4. Seasonal differences in tissue glycogen, lipid and protein in larval sea lamprey. Brain (dark bars) and carcass (light bars) were sampled from larval sea lamprey collected from Deer Creek, MI in May, June, August and September 2011 followed by measurement of tissue concentrations of (A) glycogen, (B) lipid and (C) protein. Data presented as the mean $\pm$ SEM ($n = 8$ –27 fish). In brain, mean values sharing the same capital letters were not significantly different from one another ($P \leq 0.05$), whereas data sharing the same lowercase in carcass were not statistically significant ($P \leq 0.05$).

Deer Creek, it was impossible to make predictions about how body size might have affected TFM tolerance on a seasonal basis in the present study. Although the differences in body mass we measured were statistically significant, the small ranges in body size would only lead to slight variation in rates of TFM uptake (Tessier et al., 2018). However, it is possible that in highly productive streams, where growth rates of larval sea lamprey are higher (Johnson et al., 2017), there could be substantial increases in body mass through the summer which could influence rates of TFM uptake and sensitivity. Notably, the Deer Creek population of larval sea

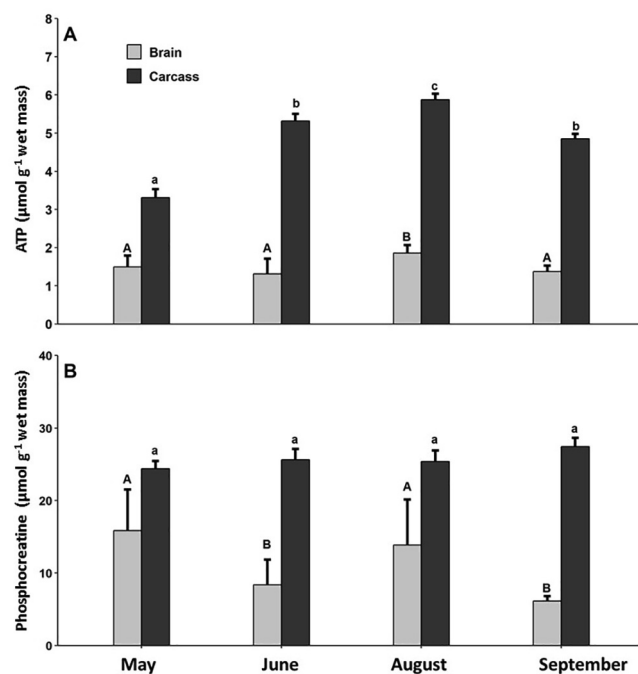


Fig. 5. Seasonal differences in tissue ATP and phosphocreatine in larval sea lamprey. Brain (dark bars) and carcass (light bars) were sampled from larval sea lamprey collected from Deer Creek, MI in May, June, August and September 2011 followed by measurement of the tissue concentrations of (A) ATP and (B) phosphocreatine. Data presented as the mean $\pm$ SEM ($n = 8$ –27 fish). In brain, mean values sharing the same capital letters were not significantly different from one another ($P \leq 0.05$), whereas data sharing the same lowercase letters were not significantly different in carcass ($P \leq 0.05$).

lamprey were found immediately downstream of a dam, where stream productivity and thus food availability would be expected to be relatively low (Brown et al., 2014).

Warmer waters increase the tolerance of sea lamprey to TFM

In addition to changes in body composition, there were marked differences in water temperature through the experiment, ranging from a low of 11.6 °C in May to a maximum of 22.2 °C in July (Table 1). MLC significantly decreased in September, when water temperatures were comparable to those measured in May, which indicates that there may be a strong positive relationship between temperature and TFM tolerance in larval sea lamprey. Using the larval sea lamprey collected from the Au Sable River, Muhametsafina et al. (2019) recently examined the effects of temperature on TFM tolerance in animals collected at the same time in summer, when tissue energy stores were uniform. Subsequent TFM toxicity tests in animals acclimated to nominal temperatures of 6,

Table 4

Seasonal differences in whole body water content and dry ash in larval sea lamprey collected from Deer Creek. Data expressed as mean $\pm$ S.E.M. Mean values sharing the same letter were not statistically significant different from one another ($P < 0.05$; $N = 8-27$).

	May	June	August	September
Water Content (% wet mass)	84.05 $\pm$ 1.16 ^a	75.87 $\pm$ 0.81 ^b	77.19 $\pm$ 0.59 ^b	79.59 $\pm$ 0.76 ^c
Dry Ash (% wet mass)	1.18 $\pm$ 0.19 ^a	1.00 $\pm$ 0.18 ^a	1.11 $\pm$ 0.18 ^a	1.35 $\pm$ 0.20 ^a

Table 5

Morphometric data of larval sea lamprey captured from Deer Creek, MI, in May, June, August and September 2011. Data presented as the mean $\pm$ 1 SEM. Data sharing the same letter (superscript) were not statistically significant from one another ($P < 0.05$). Condition Factor (CF) = $[\text{Mass}/(\text{Length})^3] \times 10^6$ (Holmes et al., 1994).

	Length (mm)	Mass (g)	³ CF	N
May	79.8 $\pm$ 0.7 ^a	0.80 $\pm$ 0.02 ^a	1.52 $\pm$ 0.02 ^a	381
June	81.5 $\pm$ 1.0 ^{a,b}	0.91 $\pm$ 0.03 ^b	1.63 $\pm$ 0.02 ^{b,c}	191
August	84.5 $\pm$ 0.9 ^{b,d}	1.01 $\pm$ 0.03 ^{b,c}	1.61 $\pm$ 0.01 ^{b,c}	130
September	87.7 $\pm$ 1.4 ^{c,d}	1.10 $\pm$ 0.05 ^c	1.56 $\pm$ 0.02 ^{a,c}	129

12 and 24 °C demonstrated that the TFM 12-h LC₅₀ was almost 2-fold higher at 24 °C compared to 6 °C (Fig. 6). Taken together, these findings strongly suggest that temperature, rather than tissue energy stores, is the primary driver behind the temporal variation in larval sea lamprey TFM tolerance.

Proposed mechanism of TFM tolerance in warmer waters

The detoxification of TFM primarily depends on the activity of the phase II detoxification enzymes which are involved in the degradation and detoxification of endogenous substances

(e.g., metabolic wastes, steroidal compounds) and exogenous toxicants (e.g., Clarke et al., 1991; Uno et al., 2012). It is well established that as external temperature increases, there are increases in the metabolic rates and enzyme activities in the tissues of poikilothermic animals such as fishes (see Schulte, 2015 for review). Likewise, the activities of detoxifying enzymes would be expected to increase in response to warmer temperatures. For instance, the rates of phase I and II biotransformation of the anti-bacterial drug triclosan by channel catfish (*Ictalurus punctatus*) increased with increases in temperature (James et al., 2012). Moreover, warmer water temperatures increased the tolerance of rainbow trout to two phenolic compounds similar to TFM, 4-nitrophenol and 2,4-dinitrophenol (Howe et al., 1994). The survival of silver perch (*Bidyanus bidyanus*), rainbow trout, rainbow fish (*Melanotaenia duboulayi*) and western carp gudgeon (*Hypseleotris klunzingerii*) exposed to phenol also increased with water temperatures (Patra et al., 2015), but only below the thermal tolerance thresholds of each fish. Interestingly, the highest temperature studied in the Deer Creek larval sea lamprey, 22.2 °C was near its thermal optima (thermal niche), which falls between 17.8 and 21.8 °C (Holmes and Lin, 1994).

The survival of an organism depends upon its physiological performance in growth, foraging, reproduction, immunity, behavior and competition, and its ability to maintain homeostasis (Pörtner and Farrell, 2008). The thermal optima (T_{opt}) of an animal represents the temperature range at which its physiological performance is maximal (Whitney et al., 2016). Physiological performance in turn relies upon O₂, which is required to generate ATP by mitochondrial oxidative phosphorylation, which is also referred to as aerobic metabolism. For this reason, the physiological performance of an organism depends upon its rate of O₂ consumption ($\dot{M}_{O_2}$) meeting its demand for ATP. In poikilothermic animals, including fishes and lamprey, the relationship between temperature and physiological performance is determined by the difference between its maximum metabolic rate (MMR) and its standard metabolic rate (SMR), which is defined as the aerobic scope (Fig. 7A; see Whitney et al., 2016 for review). In poikilothermic animals, physiological performance may improve with increasing temperature, provided that aerobic scope is sufficient to meet the ATP demands of the animal, but as temperatures increase, both SMR and MMR will also increase. At some point, the upper MMR will begin to decline, and the SMR will approach the MMR, decreasing aerobic scope and physiological performance (Schulte, 2015; Whitney et al., 2016). The temperature where performance begins to decline is the upper pejus temperature (T_{pejus}). The relationship between temperature and metabolic rate is most often determined by using respirometry to measure $\dot{M}_{O_2}$, SMR, and MMR at different temperatures (Clark et al., 2013). The

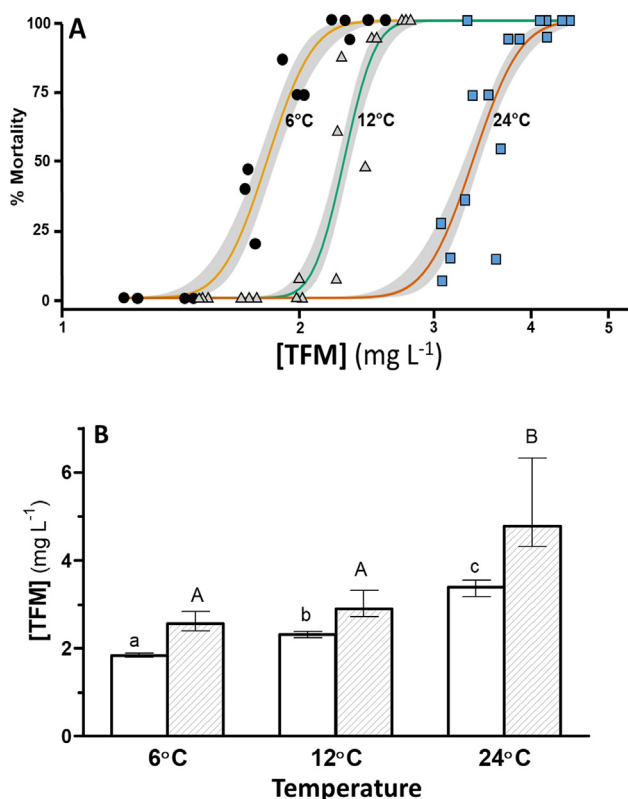


Fig. 6. Effects of temperature on the acute toxicity of TFM to larval sea lamprey. (A) Dose-response curves depicting changes in the toxicity of TFM with temperature to larval sea lamprey and (B) the corresponding 12-h LC₅₀ and minimum lethal concentration (12-h LC_{99.9}; MLC) of TFM to the same animals. Data presented as the 12-h LC₅₀ (hatched bars) or the MLC (solid bars) $\pm$ 95 % confidence interval (CI). $N = 314-315$ larval sea lamprey per temperature. Data taken from Muhametsafina et al. (2019).

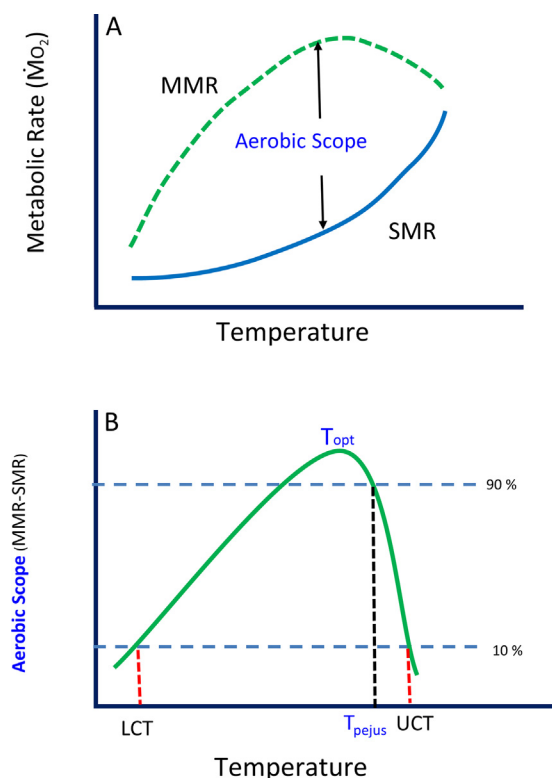


Fig. 7. Aerobic Scope and Thermal Performance Curves. **(A)** Aerobic scope is measured by first measuring standard metabolic rate (SMR) in resting, post-absorptive animals, followed by measurements of maximal metabolic rate (MMR), usually by determining M_{O_2} following exhaustive chasing. Aerobic scope, the difference between $MMR - SMR$, is then calculated at different temperatures. **(B)** The measurements of aerobic scope are then plotted against temperature, to generate thermal performance curves, which are fitted by non-linear regression or a quadratic equation as appropriate. T_{opt} : Optimum temperature for aerobic scope (performance). See text for further details.

corresponding “Thermal Performance Curves”, in which the fish’s aerobic scope is plotted against temperature (Pörtner and Farrell, 2008; Schulte, 2015), can then be used to identify the temperature where aerobic scope is maximal, T_{opt} (Fig. 7B). Thermal performance curves often skew to the right, with performance dropping off rapidly as temperatures approach the upper T_{pejus} , defined as 90% of maximum aerobic scope (Eliason et al., 2011; Chen et al., 2015), with death occurring at the upper critical temperature (UCT) or lower critical temperatures (LCT) when aerobic scope is insufficient to supply the ATP needed to sustain routine or basal activities.

For many toxicants, increases in temperature lead to increased rates of uptake, and corresponding increases in toxicity. However, toxicity is in fact a balance between rates of toxicant uptake and elimination (Fig. 8). If toxicant uptake equals toxicant elimination, the levels of the agent remain stable in the body. If elimination is less than the rate of uptake, toxicity may increase, leading eventually to death (See Ficke et al., 2007 for review). However, if elimination exceeds uptake, toxicity may decrease, which is what may be happening with larval sea lamprey exposed to TFM at warmer temperatures. Indeed, the liver could be thought of as a bilge pump on a boat, if the capacity of the bilge pump to remove water is less than the rate of water inflow, the boat will sink. If on the other hand, the bilge pump has a higher capacity, it can prevent the boat from sinking even if the boat is taking on water at a higher rate (Fig. 8). In the case of TFM, uptake may be increasing with temperature, but the enzymes involved in TFM detoxification are increasing at a proportionally higher rate. However, we would also predict

that this would only be true if the temperatures fall below T_{opt} . Beyond T_{opt} , however, the TFM sensitivity of larval sea lamprey should decrease, as aerobic scope diminishes.

Detoxification of TFM by larval sea lamprey was initially believed to be mainly through glucuronidation (Lech and Statham, 1975; Kane et al., 1994), a phase II detoxification process that adds a glucuronic acid molecule to TFM, making it more water soluble and easier to excrete. While glucuronidation in lamprey occurs at a much lower rate compared to non-target fishes (Lech and Statham, 1975; Kane et al., 1994), recent studies have detected significant levels of other TFM metabolites in lamprey exposed to TFM such as amino TFM (phase I detoxification) and sulfated TFM (phase II detoxification), along with the glucuronidated TFM (Bussy et al., 2018a,b). Thus, sea lamprey may have a higher TFM detoxification capacity than initially believed, which lends further support to our hypothesis that warmer temperatures increase detoxification rates in these fish.

While this is a tempting hypothesis, it should also be kept in mind that temperature dependent increases in metabolic rate would also be accompanied by higher rates of ventilation at the gills and increased TFM uptake, which is what is observed in larval sea lamprey. Using ^{14}C -TFM and methods from Tessier et al. (2018), we demonstrated that rates of net TFM accumulation (uptake) over the first 0.5 h of TFM exposure initially increased with temperature in larval sea lamprey exposed to the MLC of TFM (7.6 mg L^{-1} at pH 8.1, alkalinity $\sim 270 \text{ mg L}^{-1}$ as CaCO_3) at 6, 12, or 22 °C (Fig. 9A). Thereafter, the rates of net TFM accumulation dropped markedly after 1 and 2 h of exposure, suggesting that the lamprey were eliminating it more efficiently at warmer temperatures than at 6 °C, at which time the rates of accumulation were relatively stable. As a result, the internal accumulation of TFM continued to increase at 6 °C, and by 2 h exceeded the total internal TFM concentration measured at 13 °C and 22 °C (Fig. 9B). The absence of any change in internal TFM accumulation between 1 and 2 h of TFM exposure further supports our hypothesis that TFM detoxication and elimination by the lamprey were higher at warmer temperatures. The goal of future studies will be to characterize how the rates of TFM detoxification and elimination change with temperature by measuring TFM metabolite concentrations, TFM-sulfate, and TFM-glucuronide in particular, in addition to enzyme activities in lamprey exposed to TFM at different temperatures.

Implications for sea lamprey control

Residual sea lamprey that survive TFM treatment are an ongoing concern of the Sea Lamprey Control Program, and because streams are usually treated when the abundance of immediately pre-metamorphic larval sea lamprey (those most likely to undergo metamorphosis the following year) is greatest, an unsuccessful treatment can adversely affect Great Lakes fisheries in a relatively short time frame due to increased recruitment of parasitic juvenile sea lamprey (McDonald and Kolar, 2007; Barber and Steeves, 2021). Compounding the problem is that high numbers of residual sea lamprey may also necessitate re-treatment of streams, diverting financial and human resources, and delaying the treatment of other lamprey-infested tributaries. Therefore, there is value in better characterizing the conditions that most likely result in residual sea lamprey. In addition to abrupt swings in water pH, sudden storms, or evasion of TFM by larval sea lamprey, we conclude that high water temperatures can also lead to residual sea lamprey.

The greatest risk of residual sea lamprey appears to be when water temperatures are warmest during the summer, as illustrated by the discrepancy between the chart and measured MLC values we observed at higher temperatures. Using pooled MLC data from Scholefield et al. (2008), Muhametsafina et al. (2019) and the

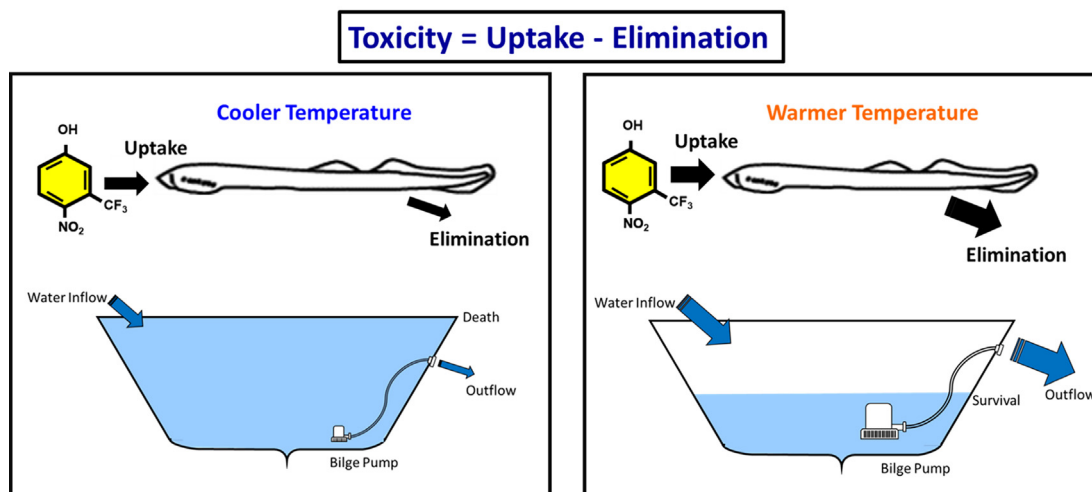


Fig. 8. Proposed working model of TFM tolerance in larval sea lamprey at cool and warm temperatures. Toxicity is the difference between rates of toxicant (TFM) uptake minus elimination. In sea lamprey, TFM is likely detoxified in the liver, which results in the generation of the less toxic metabolite TFM-sulphate, and possibly small amounts of TFM-glucuronide (Bussy et al. 2018a, b), which are more water soluble and then eliminated. In cooler waters, if rates of TFM uptake are greater than detoxification-elimination, TFM will accumulate to toxic levels causing death. Despite higher rates of TFM uptake at warmer temperatures, it is predicted that rates of TFM elimination are also increased, exceeding the rates of TFM uptake, resulting in less TFM accumulation and greater survival. In each case the liver can be thought of as a bilge pump on a boat, which removes the water (TFM) more efficiently at warmer temperatures.

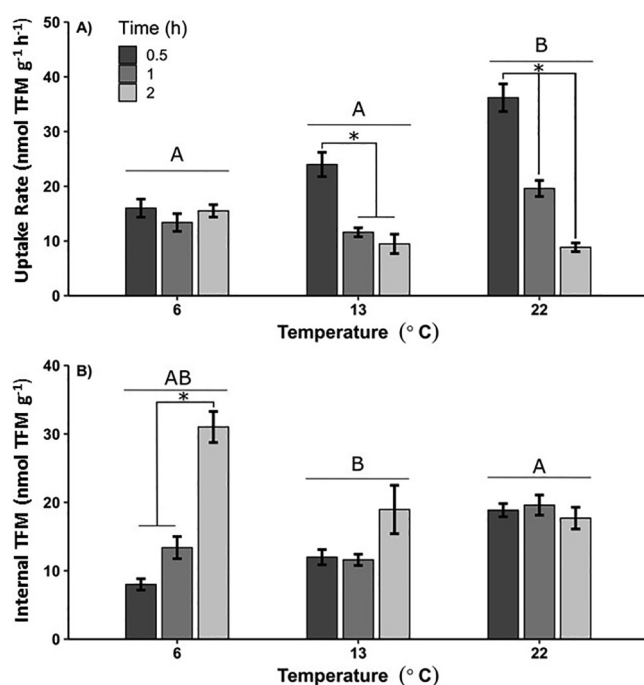


Fig. 9. Effects of temperature and time on uptake rates of 3-trifluoromethyl-4-nitrophenol. Changes in (A) uptake rates and (B) internal burden of 3-trifluoromethyl-4-nitrophenol (TFM) of larval sea lamprey acclimated to different temperatures (6, 12, and 22 °C) for 1 week. Total TFM accumulation was measured over 0.5 h (dark bars), 1 h (gray), and 2 h (light gray) intervals in different groups of lamprey exposed to radio-labelled (^{14}C -TFM) TFM at a total the 12-h $\text{LC}_{99.9}$ of TFM ($7.7 \pm 0.1 \text{ mg L}^{-1}$) in Wilfrid Laurier University well water (pH ~ 8.1 ; alkalinity $\sim 270 \text{ mg L}^{-1}$ as CaCO_3). Data are presented as the means $\pm 1 \text{ SEM}$ ($n = 11$ – 12). Letters denote a temperature effect on TFM uptake rates, while the asterisk (*) denotes a time effect within one temperature. TFM uptake rates were calculated from the accumulation of ^{14}C -TFM of TFM in the body over each time interval (see Tessier et al., 2018 for further details on methods).

present study, we compared the measured MLC values in those studies, to predicted MLC values determined from the pH-alkalinity charts published by Bills et al. (2003). All of the toxicity

tests were conducted in Lake Huron water of comparable pH (pH 7.86–8.27) and low alkalinity (74 – 90 mg L^{-1} as CaCO_3) at the HBBS. At low (5 – $8 \text{ }^\circ\text{C}$) to moderate (11 – $12 \text{ }^\circ\text{C}$) temperatures, the predicted MLC and measured MLC were comparable. However, at higher temperatures the measured MLC exceeded the predicted MLC by approximately 25 % at 17 – $18 \text{ }^\circ\text{C}$, and by more than 2-fold at 21 – $24 \text{ }^\circ\text{C}$ (Fig. 10). Even when the predicted MLC was multiplied by 1.4 times at the warmest temperatures, to more accurately reflect the highest concentrations of TFM delivered to the stream (e.g., O'Connor et al., 2017), the corresponding concentration of TFM was almost 40 % lower than the measured MLC. These comparisons further illustrate that the relative risk of residual sea lamprey is greatest when water temperatures are in the low- to mid-twenties.

The relationship between TFM tolerance and temperature has important short- and long-term implications for the Sea Lamprey Control Program in the Great Lakes. First, the much higher TFM tolerance of sea lamprey in the summer could lead to greater overall TFM requirements due to the need to use higher concentrations of TFM to minimize the risk of residual sea lamprey. When possible, it would therefore be prudent to consider applying TFM to larger rivers in the spring or the fall, when waters are cooler and the total TFM required would be lower due to the greater sensitivity of larval sea lamprey. The use of higher concentrations of TFM to achieve desired kill rates in the summer could also impact non-target species residing in the same stream, which would also receive higher doses of the lampricide, potentially increasing the risk of non-target mortality. Treating the streams in early spring, when the larval sea lamprey are more sensitive, would ensure that non-target species also receive a lower dose. Whether non-target species' sensitivity to lampricides would be higher in the spring, due to overwintering, remains to be determined.

Monitoring of stream temperatures should also be given higher priority because temporal or spatial variation in water temperatures may create “thermal TFM refuges” where the lamprey's ability to detoxify TFM may be increased sufficiently to allow survival. In such locations, additional TFM application (e.g., booster pumps) might be warranted to minimize the risk of residual sea lamprey. Greater use of stream-side toxicity tests should also be considered, especially during unusually warm periods, which would permit

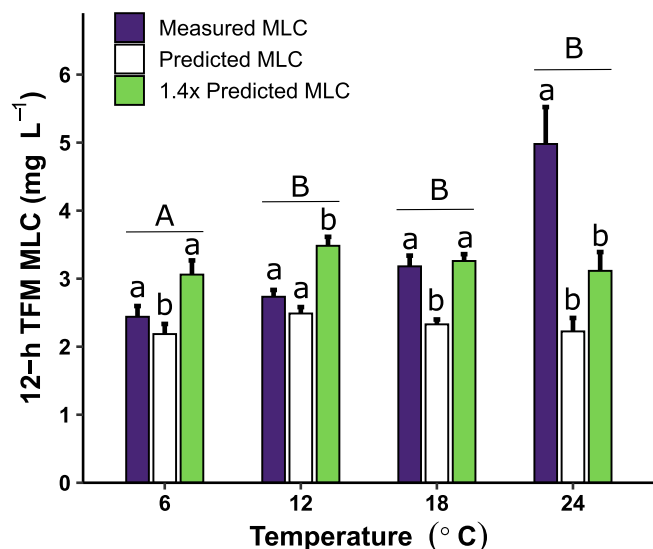


Fig. 10. Effects of temperature on the measured MLC compared to predicted MLC calculated using the pH-alkalinity model. Data comprising measured MLC (12-h LC_{99.9}) were pooled from the studies of Scholefield et al. (2008), Muhametsafina et al. (2019), and the present study (dark bars), and compared to the predicted 12-h MLC taken from the published values in Bills et al. (2003; open bars), at similar water pH (pH 7.86–8.3) and alkalinity (75–90 mg L⁻¹ as CaCO₃) values. Data were then compared to 1.4 times the predicted 12-h MLC (light gray bars) to more closely approximate TFM concentrations that would be applied in the field. Note that measured and predicted MLC values are approximately equal at 6 and 12 °C, but that measured MLC is significantly greater than predicted MLC at 18 and 24 °C, indicating that the risk of residual sea lamprey is higher in warmer waters. Even when predicted MLC is multiplied by 1.4 times, final application concentrations still fall below measured MLC at 24 °C. Data shown as the mean MLC + 1 SEM of the pooled data from each study. Upper case letters denote a temperature effect, lower case letters denote a difference between measured and predicted MLC, while the inset denotes an interaction. Bars sharing the same letter are not statistically different (two-way ANOVA, followed by Tukey's post-hoc test, $p \leq 0.05$).

treatment crews to determine MLC *in situ* and adjust TFM application rates accordingly. Finally, it may be worthwhile to conduct more comprehensive tests of how TFM toxicity is influenced by temperature and season over a range of water pH and alkalinity values, to provide treatment crews with more accurate indices of how these three variables interact, allowing them to more accurately predict MLC in the field.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We are grateful to the late Dr. D. Gordon McDonald whose intellectual contributions and encouragement were crucial in the early planning stages of this project. We thank Dr. Roger Bergstedt, Dr. Nicholas Johnson, Karen Slaght, Erik Larsen and the rest of the staff of the Hammond Bay Biological Station, United States Geological Survey, for their hospitality, patience, and support, not to mention providing us with the facilities and expertise that made these experiments possible. We also extend our sincere gratitude to Jeff Slade (retired), Aaron Jubar and their team at the Ludington Biological Station, U.S. Fish and Wildlife Service, Ludington, MI, USA, who collected the larval sea lamprey required for these experiments on numerous occasions. Nicole Thackeray provided excellent technical assistance and was partly supported by a NSERC Undergraduate

Research Assistantship. The original and previously published research reported in this paper was funded by a Great Lakes Fishery Commission research contract to MPW.

References

- Applegate, V.C., Howell, J.H., Miffed, J.W., Johnson, B.G.H., Smith, M.A., 1961. Use of 3-trifluoromethyl-4-nitrophenol as a selective sea lamprey larvicide. Great Lakes Fishery Commission Technical Report 1.
- ASTM International, 2007. Standard guide for conducting acute toxicity tests on test materials with fishes, macroinvertebrates, and amphibians. ASTM International E729-96 (Reapproved 2007), 1–22.
- Barber J., and Steeves M. 2021. Standard operating procedures for application of lampricides in the Great Lakes Fishery Commission integrated management of sea lamprey (*Petromyzon marinus*) control program. SLC 04-001.11.
- Bills, T.D., Boogaard, M.A., Johnson, D.A., Brege, D.C., Scholefield, R.J., Westman, R.W., Stephens, B.E., 2003. Development of a pH/alkalinity treatment model for the applications of the lampricide TFM to streams tributary to the Great Lakes. J. Great Lakes Res. 29, 510–520.
- Birceanu, O., McClelland, G.B., Wang, Y.S., Wilkie, M.P., 2009. Failure of ATP supply to match ATP demand: the mechanism of toxicity of the lampricide, 3-trifluoromethyl-4-nitrophenol (TFM), used to control sea lamprey (*Petromyzon marinus*) populations in the Great Lakes. Aquat. Toxicol. 94, 265–274.
- Birceanu, O., McClelland, G.B., Wang, Y.S., Brown, J.C.L., Wilkie, M.P., 2011. The lampricide 3-trifluoromethyl-4-nitrophenol (TFM) uncouples mitochondrial oxidative phosphorylation in both sea lamprey (*Petromyzon marinus*) and TFM-tolerant rainbow trout (*Oncorhynchus mykiss*). Comp. Biochem. Physiol. - C Toxicol. Pharmacol. 153 (3), 342–349.
- Birceanu, O., Sorensen, L., Henry, M., McClelland, G.B., Wang, Y.S., Wilkie, M.P., 2014. The effects of the lampricide 3-trifluoromethyl-4-nitrophenol (TFM) on fuel stores and ion balance in a non-target fish, the rainbow trout (*Oncorhynchus mykiss*). Comp. Biochem. Physiol. C. Toxicol. Pharmacol. 160, 30–41. <https://doi.org/10.1016/j.cbpc.2013.10.002>.
- Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 72, 248–254.
- Brown, L.E., Pitts, C.S., and Dun, A.M. 2014. Aquatic ecosystems. Pages 161–201 in Water Resources, J. Holden (ed.). Routledge (publ). New York.
- Brown, A.R., Gunnarsson, L., Kristiansson, E., Tyler, C.R., 2014. Assessing variation in the potential susceptibility of fish to pharmaceuticals, considering evolutionary differences in their physiology and ecology. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 369 (1656), 20130576. <https://doi.org/10.1098/rstb.2013.0576>.
- Bussy, U., Chung-Davidson, Y.-W., Buchinger, T., Li, K., Smith, S.A., Jones, A.D., Li, W., 2018a. Metabolism of a sea lamprey pesticide by fish liver enzymes part B: method development and application in quantification of TFM metabolites formed in vivo. Anal. Bioanal. Chem. 410 (6), 1763–1774. <https://doi.org/10.1007/s00216-017-0831-7>.
- Bussy, U., Chung-Davidson, Y.W., Buchinger, T., Li, K., Smith, S.A., Jones, A.D., Li, W., 2018b. Metabolism of a sea lamprey pesticide by fish liver enzymes part A: identification and synthesis of TFM metabolites. Anal. Bioanal. Chem. 410 (6), 1749–1761. <https://doi.org/10.1007/s00216-017-0830-8>.
- Chen, Z., Snow, M., Lawrence, C.S., Church, A.R., Narum, S.R., Devlin, R.H., Farrell, A. P., 2015. Selection for upper thermal tolerance in rainbow trout (*Oncorhynchus mykiss* Walbaum). J. Exp. Biol. 218 (5), 803–812.
- Clark, T.D., Sandblom, E., Jutfelt, F., 2013. Aerobic scope measurements of fishes in an era of climate change: respirometry, relevance and recommendations. J. Exp. Biol. 216, 2771–2782.
- Clarke, D.J., George, S.G., Burchell, B., 1991. Glucuronidation in Fish. Aquat. Toxicol. 20, 35–56.
- Clifford, A.M., Henry, M., Bergstedt, R., McDonald, D.G., Smits, A.S., Wilkie, M.P., 2012. Recovery of larval sea lamprey from short-term exposure to the pesticide 3-trifluoromethyl-4-nitrophenol: implications for sea lamprey control in the Great Lakes. Trans. Am. Fish. Soc. 141, 1697–1710.
- Eliason, E.J., Clark, T.D., Hague, M.J., Hanson, L.M., Gallagher, Z.S., Jeffries, K.M., Gale, M.K., Patterson, D.A., Hinch, S.G., Farrell, A.P., 2011. Differences in thermal tolerance among sockeye salmon populations. Science 332 (6025), 109–112.
- Erickson, R.J., McKim, J.M., Lien, G.J., Hoffman, A.D., Batterman, S.L., 2006. Uptake and elimination of ionizable organic chemicals at fish gills: I. Model formulation, parameterization, and behaviour. Environ. Toxicol. Chem. 25, 1512–1521.
- Eshenroder, R.L., 2009. Comment: Mitochondrial DNA analysis indicates sea lamprey are indigenous to Lake Ontario. Trans. Am. Fish. Soc. 138, 1178–1189.
- Eshenroder, R.L., 2014. The role of the Champlain Canal and Erie Canal as putative corridors for colonization of Lake Champlain and Lake Ontario by sea lamprey. Trans. Am. Fish. Soc. 143, 634–649.
- Ficke, A.D., Myrick, C.A., Hansen, L.J., 2007. Potential impacts of global climate change on freshwater fisheries. Rev. Fish Biol. Fish. 17 (4), 581–613.
- Foster, G.D., Youson, J.H., Moon, T.W., 1993. Carbohydrate metabolism in the brain of the adult sea lamprey. J. Exp. Zool. 267, 27–32.
- Great Lakes Fishery Commission, 2011. Strategic Vision of the Great Lakes Fishery Commission 2011–2020. Great Lakes Fishery Commission, Ann Arbor, MI.
- Henry, M., Birceanu, O., Clifford, A.M., McClelland, G.B., Wang, Y.S., Wilkie, M.P., 2015. Life stage dependent responses to the lampricide, 3-trifluoromethyl-4-

- nitrophenol (TFM), provide insight into glucose homeostasis and metabolism in the sea lamprey (*Petromyzon marinus*). *Comp. Biochem. Physiol. C* 169, 35–45.
- Hlina, B.L., Tessier, L.R., Wilkie, M.P., 2017. Effects of water pH on the uptake and elimination of the piscicide, 3-trifluoromethyl-4-nitrophenol (TFM), by larval sea lamprey. *Comp. Biochem. Physiol. Part - C Toxicol. Pharmacol.* 200 (October), 9–16. <https://doi.org/10.1016/j.cbpc.2017.05.005>.
- Holmes, J.A., Lin, P., 1994. Thermal niche of larval sea lamprey, *Petromyzon marinus*. *Can. J. Fish. Aquat. Sci.* 51, 253–262.
- Holmes, J.A., Youson, J.H., 1994. Fall condition factor and temperature influence of metamorphosis in sea lamprey, *Petromyzon marinus*. *Can. J. Zool.* 72, 1134–1140. <https://doi.org/10.1139/z94-151>.
- Howe, G.E., Marking, L.L., Bills, T.D., Rach, J.J., Mayer, F.L., 1994. Effects of water temperature and pH on toxicity of terbufos, trichlorfon, 4-nitrophenol, and 2,4-dinitrophenol to the amphipod *Gammarus pseudolimnaeus* and rainbow trout (*Oncorhynchus mykiss*). *Environ. Toxicol. Chem.* 13 (1), 51–66. <https://doi.org/10.1897/1552-8618>.
- Hubert, T.D., 2003. Environmental fate and effects of the lampricide TFM: A review. *J. Great Lakes Res.* 29, 456–474.
- Huerta, B., Chung-Davidson, Y.-W., Bussy, U., Zhang, Y., Bazil, J.N., Li, W., 2020. Sea lamprey cardiac mitochondrial bioenergetics after exposure to TFM and its metabolites. *Aquatic Toxicol.* 219, 105380.
- Hunn, J.B., Allen, J.L., 1974. Movement of drugs across the gills of fishes. *Annu. Rev. Pharmacol. Toxicol.* 14, 47–54.
- Ionescu, R.A., Hepditch, S., Wilkie, M.P., 2021. The lampricide 3-trifluoromethyl-4-nitrophenol (TFM) causes temporary metabolic disturbances in juvenile lake sturgeon (*Acipenser fulvescens*): implications for sea lamprey control and fisheries conservation. *Conserv. Physiol.* 9, coab069.
- James, M.O., Marth, C.J., Rowland-Faux, L., 2012. Slow O-demethylation of methytriclosan, which is rapidly glucuronidated and sulfonated in channel catfish liver and intestine. *Aquat. Toxicol.* 124–125, 72–82.
- Johnson, N.S., Swink, W.D., Brenden, T.O., 2017. Field study suggests that sex determination in sea lamprey is directly influenced by larval growth rate. *Proc. R. Soc. B* 284, 20170262. <https://doi.org/10.1098/rspb.2017.02>.
- Kane, A.S., Kahng, M.W., Reimschuessel, R., Nhamburo, P.T., Lipsky, M.M., 1994. UDP-Glucuronyltransferase kinetics for 3-trifluoromethyl-4-nitrophenol (TFM) in fish. *Trans. Am. Fish. Soc.* 123 (2), 217–222. <https://doi.org/10.1577/1548-8659>.
- Lawrie, A.H., 1970. The sea lamprey in the Great Lakes. *Trans. Am. Fish. Soc.* 99, 766–775.
- Lauff, R.F., Wood, C.M., 1996. Respiratory gas exchange, nitrogenous waste excretion, and fuel usage during starvation in juvenile rainbow trout, *Oncorhynchus mykiss*. *J. Comp. Physiol.* 165, 542–551.
- Lech, J.J., Statham, C.N., 1975. Role of glucuronide formation in selective toxicity of 3-trifluoromethyl-4-nitrophenol (TFM) for sea lamprey: Comparative aspects of TFM uptake and conjugation in sea lamprey and rainbow trout. *Toxicol. Appl. Pharmacol.* 31, 150–158.
- Lowe, D.R., Beamish, F.W.H., Potter, I.C., 1973. Changes in the proximate body composition of the landlocked sea lamprey *Petromyzon marinus* (L.) during larval life and metamorphosis. *J. Fish Biol.* 5 (6), 673–682.
- Manzon, R., Youson, J., and Holmes, J. 2015. Lamprey metamorphosis. In: Docker, M. (Ed.), *Lamprey: Biology, Conservation and Control*. Springer Dordrecht. Fish and Fisheries Science 37. pp. 139–214.
- McConville, M.B., Hubert, T.D., Remucal, C.K., 2016. Direct photolysis rates and transformation pathways of the lampricides TFM and niclosamide in simulated sunlight. *Environ. Sci. Technol.* 50 (18), 9998–10006.
- McDonald, D.G., Kolar, C.S., 2007. Research to guide the use of lampricides for controlling sea lamprey. *J. Great Lakes Res.* 33 (sp2), 20–34.
- Moore, J.W., Mallatt, J.M., 1980. Feeding of larval lamprey. *Can. J. Fish. Aquat. Sci.* 37, 1658–1664.
- Muhametsafina, A., Birceanu, O., Hlina, B.L., Tessier, L.R., Wilkie, M.P., 2019. Warmer waters increase the larval sea lamprey's (*Petromyzon marinus*) tolerance to the lampricide 3-trifluoromethyl-4-nitrophenol (TFM). *J. Great Lakes Res.* 45, 921–933.
- Niblett, P.D., Ballantyne, J.S., 1976. Uncoupling of oxidative phosphorylation in rat liver mitochondria by the lamprey larvicide TFM (3-trifluoromethyl-4-nitrophenol). *Pest. Biochem. Physiol.* 6 (4), 363–366. [https://doi.org/10.1016/0048-3575\(76\)90046-8](https://doi.org/10.1016/0048-3575(76)90046-8).
- Newman, M.C., Unger, M.A., 2003. *Fundamentals of Ecotoxicology*. CRC, Boca Raton, FL.
- O'Boyle, R.N., Beamish, F.W.H., 1977. Growth and intermediary metabolism of larval and metamorphosing stages of the landlocked sea lamprey, *Petromyzon marinus* L. *Environ. Biol. Fishes.* 2, 103–120.
- O'Connor, L.M., Pratt, T.C., Steeves, T.B., Stephens, B., Boogaard, M., Kaye, C., 2017. In situ assessment of lampricide toxicity to age-0 lake sturgeon. *J. Great Lakes Res.* 43 (1), 189–198. <https://doi.org/10.1016/j.jglr.2016.10.011>.
- Panserat, S., Médale, F., Bréque, J., Plagnes-Juan, E., Kaushik, S., 2000. Lack of significant long-term effect of dietary carbohydrates on hepatic glucose-6-phosphate expression in rainbow trout (*Oncorhynchus mykiss*). *J. Nutr. Biochem.* 11, 22–29.
- Patra, R.W., Chapman, J.C., Lim, R.P., Gehrke, P.C., Sunderam, R.M., 2015. Interactions between water temperature and contaminant toxicity to freshwater fish. *Environ. Toxicol. Chem.* 34 (8), 1809–1817. <https://doi.org/10.1002/etc.v34.8.1002/etc.2990>.
- Playle, R.C., Wood, C.M., 1989. Water chemistry changes in the gill microenvironment of rainbow trout - experimental observations and theory. *J. Comp. Physiol. B* 5, 527–537.
- Polakof, S., Panserat, S., Soengas, J.L., Moon, T.W., 2012. Glucose metabolism in fish: a review. *J. Comp. Physiol. B* 182, 1015–1045.
- Pörtner, H.O., Farrell, A.P., 2008. Physiology and climate change. *Science* 322 (October), 690–692.
- Pottinger, T.G., Rand-Weaver, M., Sumpter, J.P., 2003. Overwinter fasting and re-feeding in rainbow trout: plasma growth hormone and cortisol levels in relation to energy mobilization. *Comp. Biochem. Physiol. Part B* 136, 403–417.
- Rovainen, C.M., 1970. Glucose production by lamprey meninges. *Science* 167, 889–890.
- Rovainen, C.M., Lemcoe, G.E., Peterson, A., 1971. Structure and chemistry of glucose-producing cells in meningeal tissue of the lamprey. *Brain Res.* 30, 99–118.
- RStudio Team, 2018. *RStudio: Integrated Development for R*. RStudio, PBC, Boston, MA. <http://www.rstudio.com/>.
- Scholefield, R.J., Slaght, K.S., Stephens, B.E., 2008. Seasonal variation in sensitivity of larval sea lamprey to the lampricide 3-trifluoromethyl-4-nitrophenol. *North Am. J. Fish. Manag.* 28 (5), 1609–1617. <https://doi.org/10.1577/M06-178.1>.
- Schulte, P.M., 2015. The effects of temperature on aerobic metabolism: towards a mechanistic understanding of the responses of ectotherms to a changing environment. *J. Exp. Biol.* 218, 1856–1866.
- Siefkes, M.J., 2017. Use of physiological knowledge to control the invasive sea lamprey (*Petromyzon marinus*) in the Laurentian Great Lakes. *Cons. Physiol.* 5 (1), 2017, cox031. <https://doi.org/10.1093/conphys/cox031>.
- Smith, B., Tibbles, J., 1980. Sea lamprey (*Petromyzon marinus*) in Lakes Huron, Michigan, and Superior: history of invasion and control, 1936–78. *Can. J. Fish. Aquat. Sci.* 37, 1780–1801.
- Soengas, J.L., Strong, E.F., Andres, M.D., 1998. Glucose, lactate and β -hydroxybutyrate utilization by rainbow trout brain: changes during food deprivation. *Physiol. Zool.* 71, 285–293.
- Sprague, J.B., 1969. Measurement of pollutant toxicity to fish I. Bioassay methods for acute toxicity. *Water Res.* 3, 793–821.
- Swink, W.D., Johnson, N.S., 2014. Growth and survival of sea lamprey from metamorphosis to spawning in Lake Huron. *Trans. Am. Fish. Soc.* 143, 380–386.
- Tessier, L.R., Long, T.A.F., Wilkie, M.P., 2018. Influence of body size, metabolic rate and life history stage on the uptake and excretion of the lampricide 3-trifluoromethyl-4-nitrophenol (TFM) by invasive sea lamprey (*Petromyzon marinus*). *Aquat. Toxicol.* 194, 27–36. <https://doi.org/10.1016/j.aquatox.2017.10.020>.
- Uno, T., Ishizuka, M., Itakura, T., 2012. Cytochrome P450 (CYP) in fish. *Environ. Toxicol. Pharmacol.* 34, 1–13.
- Whitney, J.E., Al-Chokhachy, R., Bunnell, D.B., Caldwell, C.A., Cooke, S.J., Eliason, E.J., Roger, M., Lynch, A.J., Paukert, C.P., 2016. Physiological basis of climate change impacts on north American inland fishes. *Fisheries* 41, 332–345. <https://doi.org/10.1080/03632415.2016.1186656>.
- Wickham, H., 2016. *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag, New York. <https://ggplot2.tidyverse.org>.
- Wilkie, M.P., Bradshaw, P.G., Joanis, V., Claude, J.F., Swindell, S.L., 2001. Rapid metabolic recovery following vigorous exercise in burrow-dwelling larval sea lamprey (*Petromyzon marinus*). *Physiol. Biochem. Zool.* 74, 261–272.
- Wilkie, M.P., Holmes, J.A., Youson, J.H., 2007. The lampricide 3-trifluoromethyl-4-nitrophenol (TFM) interferes with intermediary metabolism and glucose homeostasis, but not with ion balance, in larval sea lamprey (*Petromyzon marinus*). *Can. J. Fish. Aquat. Sci.* 64 (9), 1174–1182.
- Wilkie, M.P., Hubert, T.D., Boogaard, M.A., Birceanu, O., 2019. Control of invasive sea lamprey using the piscicides TFM and niclosamide: Toxicology, successes & future prospects. *Aquat. Toxicol.* 211, 235–252.
- Wilkie, M.P., Tessier, L.R., Boogaard, M., O'Connor, L., Birceanu, O., Steeves, T.B., Hubert, T.D., and Sullivan, W.P. 2021. Lampricide Bioavailability and Toxicity to Invasive Sea Lamprey and Non-Target Fishes: The Importance of Alkalinity and the Gill Microenvironment. *J. Great Lakes Res.* 47, S407–S420.
- Youson, J.H., 2003. The biology of metamorphosis in sea lamprey: Endocrine, environmental, and physiological cues and events, and their potential application to lamprey control. *J. Great Lakes Res.* 29, 26–49.